

The University of Chicago

STUDIES ON THE CRYSTALLINE
LENS

A DISSERTATION SUBMITTED TO THE
FACULTY OF THE DIVISION OF THE BIO-
LOGICAL SCIENCES IN CANDIDACY FOR
THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF PHYSIOLOGY

1935

By
EVERETTE I. EVANS

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THE RELATION OF THE PARATHYROID GLAND TO CATARACT

EVERETTE I. EVANS*

CHICAGO

A chemical study was made of a number of normal dogs' lenses and of parathyroid cataract lenses experimentally produced in dogs. Analytical data on percentage of water, calcium, magnesium, potassium, phosphorus, and silicon thus obtained are presented and compared with other data on the subject. An increase in calcium and a decrease in potassium was found similar to that noted in senile cataract. An extensive review of the literature is presented. From the Department of Physiology, University of Chicago.

Recent literature on the subject of etiology of cataract is evidence that considerably more attention is being paid to this particular phase of the cataract problem than to surgical treatment alone. Prior to this century but little work had been done relative to the pathological physiology of the crystalline lens. And as Kirkpatrick¹ has pointed out, the extent of our knowledge with regard to the causes of the disease and the effectiveness of treatment once the disease has commenced, depend in a large measure upon the investigations into the physiological nature of the true cause of the disease.

Edward Jackson² has only lately remarked editorially of the possible connection of parathyroid gland dysfunction and cataract. It was because of reports of numerous observations of clinicians and laboratory workers of the apparent significance of the parathyroid gland in the etiology of cataract that this study was undertaken.

Literature

Fischer and Triebenstein³ report that 88.2 percent of patients examined by them showed latent tetany, and

these workers concluded that senile cataract was associated with parathyroid deficiency. Tron⁴, Polichova⁵, and V. Pellathy⁶ all investigated serum calcium of senile cataract patients and found only slight differences from the normal. Greppin⁷ thought tetany was due to parathyroid deficiency and states that 50 percent of his cataract cases were associated with tetany. Erdheim⁸, Possek⁹, Shiøtz¹⁰, Hiroishi¹¹, Luckhardt and Blumenstock¹², and Luckhardt and Eiseman¹³ have all recognized the connection between parathyroid deficiency and cataract. In a later paper, V. Pellathy¹⁴ has described the pathological anatomy of the cataractous lens brought about by previous parathyroidectomy.

It was Burge¹⁵ in this country who first examined the lenses of senile cataract patients for their inorganic constituents and whose work remains unchallenged in these respects. Burge called attention in this paper to a decrease of potassium from 38.8 percent ash of normal lens to 9.8 percent in cataractous lens with a corresponding increase in calcium content from a negligible quantity in normal lens to 12.5 percent in the cataractous lens. Having had no opportunity to make sodium and

* T. J. Williams Fellow in Physiology.

magnesium analyses on ash of normal human lenses, Burge made the assumption that normal pig lenses have about the same sodium and magnesium content as normal human lenses, so analyzed, for comparison with human cataractous lenses, normal pig lenses for sodium and magnesium. Having made this assumption, Burge showed an increase of sodium from 6.67 percent in normal pig lens to 25.06 percent in cataractous human lens. Burge's figures also show an increase in magnesium in human cataractous lens of 8.0 percent as compared to normal pig lens of 1.2 percent. In a later paper, Burge¹⁶ suggested from an analysis of these data two factors for the production of cataract; namely, (a) modification of lens protein and (b) radiation of short wave lengths by which the modified protein can be coagulated. Indeed Burge¹⁷ was able to produce cataract in goldfish by means of ultraviolet irradiation if these fish were allowed to stay in a bath of 0.8 percent calcium chloride solution or sodium silicate solution for ten days, with subsequent irradiation.

Adams¹⁸ has shown, however, that the mere increase in calcium content of the blood is not sufficient to cause cataract, nor does a persistent high blood calcium accelerate the development of naphthalene cataract. She has demonstrated that calcium salts acting on fresh ox lenses do not cause opacity except in unphysiological concentrations, nor do they act as sensitizers in the production of cataract by ultraviolet irradiations. Her figures for analysis of ash of normal and senile cataractous lenses demonstrate the same phenomena as did Burge's, that there is an increase in calcium content over that of the normal lens, with a decrease in potassium content.

The data of Adams on effect of ultraviolet light on alpha and beta crystallin of the lens in which these proteins show a certain sensitivity to solutions of calcium salts when irradiated are interesting and are quite in line with the reports of Hinrichs¹⁹ that ultraviolet radiation produces a slight opacity in the lens proteins which is enhanced by

presence of salts, particularly calcium, at time of exposure.

From the above it may be seen that these workers are nearly all agreed that there is a demonstrable change in calcium content in the senile cataractous lens and that lens proteins show a sensitivity toward calcium salts. Furthermore, the clinical observations of Weseley²⁰ who concluded that the crystals in cataract are calcium phosphate; Braun²¹, that crystals in lens of congenital cataract cases were probably CaCO_3 ; and Boente²², who found crystals of CaCO_3 in lens of senile cataract patient, all point out the fact that in some obscure way salts of calcium or the calcium ion are associated with cataract.

It was in the light of the results of the above investigations that the production of parathyroid cataract in dogs was undertaken, making again analyses of normal lens for comparison with analyses of cataract lens produced under carefully controlled laboratory conditions. It was thought that if some comparisons might be made between the experimentally produced parathyroid cataract and the senile cataract as observed in the clinic, some real advance might be made into the understanding of the causes of senile cataract.

Experimental

Only healthy dogs were used in this experiment. After an examination of both eyes of the dog had been made and a control period in which several determinations were made on various inorganic ions of the blood, including calcium, magnesium, sodium, potassium and chlorides, the dogs were submitted to a bilateral thyroparathyroidectomy under morphine-ether anesthesia. The dogs were allowed to convalesce, with daily administration of 1.5 gm. calcium lactate per Kg. per day, which has been shown by Luckhardt and Goldberg²³, Salvesen²⁴ and later by Dragstedt²⁵ to keep thyroparathyroidectomized dogs free from tetany. On the sixth and seventh days, no treatment was given of calcium lactate, and if then the animals did not develop parathyroid tet-

any, they were considered to have accessory parathyroids which had not been removed by operation, and were therefore not used in this study. Furthermore, only those animals which developed, in course of test periods for parathyroid deficiency, extreme signs of tetany, were used in this study, for Luckhardt and Blumenstock¹² noted a direct relation between the severity of the tetany and the rate of development and extent of the cataract.

The daily dose of 1.5 gm. calcium lactate per Kg. body weight was maintained as long as animals showed tetanic tendencies, which was usually for 40 to 60 days after operation, as shown by Luckhardt and Goldberg²³. Thereafter, the dose was withdrawn and the animals kept on a maintenance diet of bread and meat, care being taken not to give too much of the latter.

After 6 to 8 weeks, examinations of the lenses of the surviving animals were made with the Gullstrand slit-lamp and more often with the ophthalmoscope, by ophthalmoscopy at a distance and by oblique illumination. These examinations were made to the time the animals were sacrificed for analyses of lenses.

In connection with another study, blood samples were taken during the survival period and will be reported in another paper²⁶. The dogs at time of death had been in state of "parathyroid survival" for a period of 11 to 24 months.

The lenses were excised as soon as possible after the animals were killed with an intrapleural injection of CHCl_3 . Some of these lenses were used for respiration experiments in the Warburg apparatus; the others were escapulated, weighed and dried in a tared platinum crucible to constant weight at 85°C . After the respiration experiments, the other lenses were treated in the same manner.

Analytical procedure

The lenses were removed from the eyes immediately after anæsthetizing and placed at once into a weighed platinum crucible. The weight was taken at

once to 1 mg. The lenses were then dried in an electric oven at 85°C . to constant weight, care being taken not to heat to too high a temperature as they char very easily. After weighing, the dried lenses were ashed as sulphates, allowing 1 cc. of concentrated H_2SO_4 to 6 lenses. Heating was carried out at a low temperature until the lenses were in solution, then the sulphuric acid was driven off by increasing the temperature; finally the temperature was raised until the ash was white, care being taken that the ash was not subjected to dull red heat long enough to risk the chance of some loss of SO_3 . The ash was weighed after desiccation to 0.1 mg.

The residue was dissolved as completely as possible in water. A drop of methyl orange was added to make sure no sulphuric acid was left. A little hydrochloric acid was added to dissolve the remaining residue. The solution was then filtered quantitatively into a volumetric flask and made up to volume, allowing 4 lenses to each 10 cc. The filter paper was ashed in a weighed platinum crucible, and weighed again for silicon. Determinations were carried out on aliquots of this solution, using 1 cc. for phosphorus, 1 cc. for potassium, 5 cc. for calcium, 5 cc. for sodium, and 4 cc. of the solution from the calcium precipitation for magnesium.

The calcium was determined by the technique of Kramer and Tisdall²⁷, slightly modified. After the addition of ammonium oxalate the pH was adjusted to brom-cresol purple. The precipitated and centrifuged calcium oxalate was washed twice with a saturated ammonium oxalate solution in 0.5 percent ammonium hydroxide. The third washing was with 2 percent ammonium hydroxide. Titrations were made with $n/200\text{ KMnO}_4$. The permanganate factor was redetermined in every experiment by means of standard sodium oxalate.

For phosphorous the technique of Fiske and Subarrow²⁸ was used, care being taken to use the same molybdate solution (I) in the standard and unknown. A compensation for trichloro-

acetic acid is not needed, and none was used.

The sodium was determined by the technique of Kramer and Gittleman²⁹ with slight modifications as worked out in Dr. A. B. Hastings' laboratory. After adding the potassium antimonate reagent, the sides of the tube were rubbed with a pyrex glass rod until a good heavy precipitate was formed. Then 4 cc. of 80 percent redistilled alcohol was added drop-wise from a burette with constant stirring. The rod was rinsed down with 30 percent alcohol and this same rod was replaced in the tube before titrating. The tube was covered and after standing 1 to 1½ hours, was centrifuged for 10 to 15 minutes. It was then inverted and drained on a filter paper for 5 minutes. The titration was carried out in the centrifuge tube, the volume having been made up to 10 cc. before titrating.

The technique of Kramer and Tisdall³⁰ with modifications by Dr. A. B. Hastings was used for potassium. After precipitation was complete the volume was made up to 5 cc. with water and 0.5 cc. of a 2 percent suspension of caprylic alcohol in water added. The tube was rotated until the precipitate

just began to rise and centrifuged at once for 10 to 15 minutes. The supernatant fluid was poured off by tilting the tube carefully so as not to let the precipitate run down the side of the tube. The tube was then drained and wiped with a filter paper and the washing repeated twice more. Very fine tipped centrifuge tubes were used to diminish the danger of losing precipitate.

Magnesium was determined on 4 cc. of the supernatant fluid from the calcium by the method of Denis³¹.

The silicon was determined by weighing the insoluble residue.

Results

It may be stated that throughout the survival period, even after the parathyroidectomized animals were free from any signs of tetany, the blood calcium level is low while the blood inorganic phosphorous is high.

Table I is constructed from data of Burge and our data on lenses of normal and parathyroidectomized dogs.

Calcium content

It appears from our data that the calcium content of the normal lens is about

Table I
BURGE

	Avg. Wt. Dry Lens Mgms.	Avg. Wt. Ash One Lens Mgms.	Percent K Ash	Percent Ca	Percent Mg	Percent Na	Percent Si
Normal Adult Human	58.99	1.40	38.80	?	?	?	0
Normal Adult Pig	137.70	3.40	34.30	0.08	1.20	6.67	0
Embryo Human	15.47	0.25	30.80	?	?	?	0
Cataract Human U. S.	34.42	0.58	9.80	12.50	8.00	23.82	0
Cataract Human India	92.30	1.52	5.81	6.00	1.60	25.06	3.63

EVANS

	No. Lenses	Avg. Wt. One Lens Wet Mgms.	Avg. Wt. One Lens Dry Mgms.	Ash One Lens Mgms.	Per- cent H ₂ O	Ca Ash	Per- cent P	Per- cent Na	Per- cent K	Per- cent Mg	Per- cent Si
Normal Dogs	6	454	179	2.8	60.5	0.7	1.7	17.6	26.7	0.8	0
Normal Dogs	8	340	129	2.7	62.1	1.4	2.5	21.8	30.3	—	0
Normal Dogs	13	537	213	4.3	60.3	1.2	4.5	10.0	29.8	1.0	0
Normal Dogs	23	469	183	4.4	61.0	1.5	4.7	9.4	31.3	0.8	0
Cataract P.T.	10	427.5	149.6	2.8	64.0	28.7	1.1	17.4	21.6	2.0	0

1.2 percent of the total ash. No figures could be found for comparison with dog lens, but Burge showed that the amount of calcium in normal human lens is so small as to be impossible to analyze. In the normal pig lens, he reports a value of 0.08 percent of ash as calcium.

There seems to be established the clear fact that in senile cataract there is an increase in calcium content over that of the normal lens. Burge's and Adams' figures both show such an increase while from our data it may be seen that the calcium content is increased to twenty-five times that of the normal lens.

Potassium

The potassium content of normal dog lens is averaged as 29.5 percent of ash, comparing favorably with figures of Burge showing for normal human lens a potassium content of 38.8 percent of ash.

The potassium content of "parathyroid" cataract shows a decrease to 21.8 percent, a decrease of 8.0 percent of ash which we believe to be significant and which checks again Burge's data for human senile cataract, potassium content of 9.80 percent of ash, a decrease of 29.0 percent of ash from normal. Although one determination in our "normal" series is 26.7 percent potassium of the total ash, the general potassium content of the 50 normal lenses is well above 29.5 percent so that we feel the data do illustrate a real decrease in potassium content in the cataractous lenses.

Sodium

It is not thought that there is a demonstrable change in sodium content of the "parathyroid" cataract; it appears that there is some normal variation in sodium content of the normal lens.

However, Burge's figures show an increase in sodium content of ash of senile cataract lens of about 17.0 percent. In this respect our data do not check those of Burge.

Phosphorus and magnesium

There has been no increase in phosphorus content of cataractous lens as was expected, but, if anything, a slight decrease to 1.1 percent of ash of cataractous lens. This decrease is not considered significant.

The magnesium content appears to be the same in both types of lens, which data do not check those of Burge, who demonstrated an increase of about 6.0 percent of ash in senile cataractous lens over the normal lens ash.

Silicon

In no case have we been able to detect the presence of silicon in any lenses, either cataractous or normal. This is in line with data of Burge on normal and cataractous adult human lens, but he did show a silicon content of ash of 3.63 percent in cataractous lens from India.

Water content

The water content of normal dog lens is about 61.0 percent. The water content of the cataractous lens shows an increase to 64.0 percent. No figures could be found for comparison with dog lens.

It may be stated here that should a question arise as to why these cataractous lenses were grouped together for analyses, that our reason for doing so was to increase the reliability of our analyses and to show gross general changes rather than select individual changes. Adams¹⁸ and Salit³² both agree that there is a wide variation in calcium content of senile cataractous lenses, and for this reason we believed it to be to the benefit of our experiment to group the lenses for analyses rather than attempt to analyze minute quantities of the several inorganic salts in individual lenses.

Discussion

From the above data, the striking similarity of changes in mineral content of both senile cataractous lenses and cataracts induced by parathyroidectomy is evident, and one is inclined to conclude that the causes of each

type of cataract in some obscure manner may be substantially the same in each case. At least, we feel that these results should focus attention upon a more serious consideration of the rôle the parathyroid gland dysfunction might very probably play in the etiology of senile cataract. In this instance, we do not mean to imply that (as Burdon-Cooper³³ cautions against) to the increase in mineral content of lens alone is to be attributed the cause of cataract, but we do maintain that when in an experimental manner this increase in mineral content is brought about only by excision of the parathyroid glands that some serious notice should be taken of the rôle of these glands in the maintenance of normal conditions of the lens proteins. It is well understood by the authors that these changes in the lens may be caused not by the lack of the parathyroid hormone itself, but perhaps upon the lack of the hormone on the proper functioning of other systems in the animal body which in their normal activity keep the lens proteins in a stable state.

It is further evident from the above data on parathyroid lens cataract that there is not a deposition of $\text{Ca}_3(\text{PO}_4)_2$

stated again that in the parathyroidectomized dog the blood calcium is persistently low during the survival period while the inorganic phosphorous in a majority of cases is persistently high, so we feel that production of parathyroid cataract is in no direct way dependent upon the calcium content of the blood.

Because of the obvious importance of oxygen consumption in determining the metabolism of the lens (see Karr and Tassman³⁵, Schmerl³⁶, and Kronfeld and Bothman³⁷) and the stability of the glutathione system, the oxygen consumption experiments were made on several of the cataractous lenses from parathyroidectomized animals. It is unfortunate that these experiments had to be made at 20° C., so perhaps the only value of these data on oxygen consumption lies within the experiment itself and cannot be used for comparison with other data. It is believed, however, that by applying the proper temperature coefficient these data could be compared with other data taken at 37° C. The nitro-prusside test for presence of glutathione or cysteine was not made on these lenses as they were to be used for subsequent analyses.

Table II

		cu. mm. O_2 per hour @ 20° C.
Parathyroid Cataract	(11 mos.)	
	555 mgms.	1.67
	551 mgms.	4.83
Parathyroid Cataract	(11 mos.)	
	486 mgms.	3.2
	423 mgms.	3.4
Parathyroid Cataract	Very Early Cataract	2.3
Normal Lens	No. 2	1.06
Normal Lens	No. 1	3.4
	No. 2	5.0

in the lens after thyroparathyroidectomy as Greenwald³⁴ has suggested. It is thereby shown that the lens does not act as a depot for the increased retention of calcium and phosphorus which follows upon parathyroidectomy in the dog as shown by Greenwald.

Relative to statement of Adams¹⁸ that "an experimentally produced persistent high blood calcium in rabbits does not cause cataract," it may be

These experiments were made in the Warburg apparatus, care being taken that no injury was made to the lens as Schmerl³⁶ had cautioned that a slight injury to lens capsule would markedly increase the oxygen consumption.

It is believed that these data check fairly well those of Kronfeld and Bothman³⁷ for rabbit lens if a temperature coefficient is applied. However, they may be a little high.

Schmerl¹³⁶ believes that in the beginning stages, every kind of lesion in the lens is characterized by an increased oxygen consumption. In no "parathyroid" lens does this seem to hold true and in a preliminary study on naphthalene cataract (early stage) this thesis is not borne out.

Of the lenses reported in this study of oxygen consumption, it cannot be said that they were in a state of late cataract, but rather at about a midpoint. The cataracts were of the typical aparathyroid type, an abundance of fine, flaky, crystalline deposits being present in the cortex, with a few powdery opacities in the nucleus. The lens had not become hard except in one case, and this case could not be used for oxygen consumption experiment. Undoubtedly, in a late cataract, the internal oxidative system is badly damaged and one should expect a decreased oxygen consumption in this type of lens, but it appears from these data that in early cataractous changes, the internal oxidative system is still in quite good order to carry on the metabolism of the lens.

Burdon-Cooper³³, in discussing the close relation of surface tension of aqueous humour in cataract and the diminished osmotic pressure and freezing point of urine suggests a kidney deficiency as the cause of cataract. Greenwald³⁸ has made the suggestion that there is an interference with renal function after parathyroidectomy due to decreased calcium concentration of the blood, with a decreased excretion of sodium and potassium. It may be stated in this connection with data on blood concentrations of sodium, potassium and chlorides on parathyroidectomized dogs in survival period show concentrations of these ions in accepted normal amounts, so that it does not appear from these data that there is a severe disturbance in renal function in parathyroidectomized animals.

Burger and Schlomka³⁹, Salit³², and Jess⁴⁰ all state that with increasing age there is a decrease in water content of the lens and that there is a decrease in water content of the senile cataractous lens. Salit has suggested that this de-

crease "may be a dehydration of lens protein." In this study with "parathyroid" cataractous lenses, there was found no decrease in water content but rather a slight increase. The variations in water content of the normal dog lens are slight and this increase of 2.0 percent water we believe to be significant. It may indicate a change in the permeability of the lens capsule which permits the calcium to enter causing first swelling with subsequent opacification (T. J. Williams).

The importance of the lens protein, effects of changes in osmotic pressure of the aqueous humour, and many other points have not been considered in this discussion because we believe existing data on the parathyroid cataract and other forms of cataract are not sufficient to warrant their discussion in this paper. The possibility of formation of complex salts of proteins with ions of electrolytes seems to have been established with the work of Northrup and his associates⁴¹ and we are of the opinion that data should be secured on the action of salts of calcium and magnesium with the proteins of the crystalline lens. It may be that in cataract we are dealing with complex salt formation of the crystallines with calcium.

Gortner and his associates⁴² have done considerable work on the question of the state of water in plant protoplasm. From a casual study of this problem of the relationship of proteins to water in the cell, the writers believe that it might be profitable to make a study of the state of water in the normal and cataractous lens. To date, as far as we are aware, no such attempt has been made to correlate these cataractous changes with any change of state which might occur in the water of the lens.

We wish to take this opportunity to thank Dr. Luckhardt, at whose suggestion this investigation was carried out, for his interest and help in the course of the experiments.

Summary

Analytical data on the quantity of water, calcium, magnesium, potassium,

phosphorous and silicon in the normal lens (dog) and "parathyroid" cataractous lens (dog) are presented and compared with other data on the subject. That there is an increase in calcium and a decrease of potassium in the parathyroid cataractous lens seems to be established with close correlation with senile cataract changes. Data on oxygen consumption at 20° C. of catarac-

tous and normal lens (dog) are given and discussion of their significance is made.

Because of the apparent correlation of existing data on the two types of cataract, the authors feel that serious consideration should be given the parathyroid gland dysfunction in the etiology of senile cataract.

University of Chicago.

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The role of tetany in parathyroid cataract

EVERETTE I. EVANS

CHICAGO

Young dogs kept on a special bread-sour-milk-liver diet for a month after weaning were thyroparathyroidectomized. Tetany was not seen to occur in any of the young dogs so treated. Examination of the lenses of these animals indicated that there was a characteristic change in the lenses of the young, parathyroidectomized dog, very similar to that described by Goldmann. The chief difference between Goldmann's experiments and these is that Goldmann noted no lenticular change if tetany was not allowed to occur. In these dogs characteristic lens changes occurred although no tetany was seen. Emphasis is made on the possible role of toxins in the development of the parathyroid cataract. From the Department of Physiology, University of Chicago.

Introduction

In an earlier paper (Evans and Kern, 1931), comparison was made of the inorganic content of experimentally produced parathyroid-cataractous dog lenses and that of clinical senile human cataractous lenses. It was shown that the calcium content of the ash of "parathyroid-cataractous dog lens" is approximately 25 times that of normal dog lens. In the present communication, the author desires to report the occurrence and describe the lenticular degeneration leading to opacity in five young parathyroidectomized dogs in which tetany never occurred.

Literature

The earlier observations of Luckhardt and Blumenstock (1923) that animals which have survived complete parathyroidectomy eight months or more, show bilateral cataracts have been confirmed (Goldmann, 1929; Evans and Kern, 1931; Evans, 1933). Later Eiseman and Luckhardt (1927) demonstrated that, contrary to the opinion of some clinicians, convulsions (oil of wormwood and strychnine), *per se*, would not produce cataracts. Further, Eiseman and Luckhardt were able to show that no cataracts appeared in dogs treated in such a way that there existed a state of low blood calcium or high blood inorganic phosphorus.

Goldmann (1929) made an extensive study of parathyroid cataract in a group of 40 rats and 2 dogs. One dog (Bobby) was given sufficient calcium lactate so that no symptoms of parathyroid deficiency were observed. When the cal-

cium lactate in the diet was diminished and tetany allowed, Goldmann noted changes in the lenses of this dog two days later. Further, he found that whenever tetany was allowed to occur, new lens changes followed shortly thereafter. In another dog (Fifi), the tetany was not controlled so closely, with the result that the dog suffered numerous attacks.

Goldmann concluded from his study on tetany cataract that "Ohne muskeltetanische Symptome keine Cataract." Further, the extent of the opacities appeared to be in direct relation to the number of attacks of tetany suffered by the parathyroidectomized animal. If the tetanic seizures were suppressed by administration of calcium lactate, so also were the lenticular changes suppressed. Hyperemia of the ciliary vessels enhanced the lens changes. From Goldmann's paper it appears that he did not believe that the convulsion of the tetany, *per se*, caused the lenticular opacities, but rather that they were the result of the general toxic disturbance, and toxins had diffused from the blood vessels into the aqueous humor and then attacked the lens fibers.

Because it is apparent that there still exists some question as to the actual role of the tetany in the etiology of clinical parathyroid (postoperative tetany) cataracts the author desires to stress the point that *in the experiments now being reported no tetany was ever observed* in the young dogs. O'Brien (1932) has reviewed the literature pertaining to this question and it need not be repeated here.

Experimental data

The dietary control of parathyroid tetany in young dogs by bread-and-sour-milk diet will be outlined elsewhere (Evans 1934^a) so only a brief mention will be made of the experimental procedure.

Eight young dogs were placed on a diet of sour milk, bread, and liver immediately after they were weaned. This diet was continued for 30 days, when the dogs were completely thyroparathyroidectomized. Death from tetany occurring in only one dog, this diet was continued for 40 days after the operation and after this the living dogs were placed on the regular laboratory diet of bread and meat, plus 100 gm. of sour milk daily. After the 75th day of survival the sour milk was omitted.

At no time during the period of this dietary regime was tetany seen to occur in any of these 7 dogs. It is apparent that these animals had been completely parathyroidectomized because immediately after the operation the blood calcium dropped from the normal level of 10 mgm. percent to the usual tetany level of 5 to 6 mgm. percent. Further, the blood inorganic phosphorus rose to the tetany level of 10 mgm. percent. It was evident to the writer that here was afforded a means of controlling post-operative parathyroid tetany in young dogs. It was hoped that the customary lenticular changes following parathyroidectomy would not occur in these dogs, but such hopes were not realized. Five of the seven dogs showed lenticular changes as a result of parathyroid deficiency.

Almost daily examinations were made of the lenses of these animals, with the ophthalmoscope at a distance and with a magnifying glass and transmitted light. It was intended that slit-lamp examinations should be made and the necessary steps had been taken to do this. Unfortunately, although the animals had survived the operation for 120 to 150 days, before the slitlamp examinations could be made all the dogs contracted a severe form of distemper and had to be killed. In this report, therefore, we are dealing with lenticular changes that began approximately one

month after the operation and continued to develop for four months.

Six adult dogs were then given this same dietary treatment before and after thyroparathyroidectomy. Of this adult group only one survived long enough (57 days) for lenticular changes to become evident. This dog had but one attack of tetany.

The lenticular changes in these dogs are described in the discussion of the results obtained from this study. When any of the five young thyroparathyroidectomized dogs became so ill from distemper that recovery was deemed impossible, the animal was killed by the intravenous injection of 50 mgm. sodium cyanide. The eyes were excised within 2 or 3 minutes, the lenses removed, excapsulated, and glutathione determinations were made immediately on the sulphosalicylic extracts of these lenses. The data on glutathione are reported elsewhere (Evans 1934^b). In every case, however, the glutathione content of these lenses was 50 to 70 percent lower than that found in normal dog lenses; that is to say, the glutathione content of these cataractous lenses was about 0.150 percent, while, by the methods used, the normal dog lens showed consistently a glutathione content of from 0.380 to 0.390 percent.

Lenticular changes

In our earlier study of the biochemical nature of postoperative-tetany cataract in the dog, a complete examination of the nature of the earlier degenerative process in the lens was not made. We were interested at that time in the gross pathology as indicated by the increase in calcium content of the lens. In the present study, however, the writer took particular care to begin ophthalmoscopic examinations of the lenses of the young parathyroidectomized dogs immediately after the operation. In the 5 young dogs in which lenticular degeneration was noted, these changes were so strikingly uniform that a description of the lenses of only one dog will suffice. In every case the same pathology was observed in a more or less advanced degree.

In one case, not considered hereto-

fore, an apparent total thyroparathyroidectomy was done in a young dog treated in the above manner. However, when examinations were made of the blood calcium and phosphorus after the operation, there was no change from the normal for a period of at least five months following the operation. This dog, then, may be considered to have had accessory parathyroids. In the five months after the operation, there was some evidence of thyroid insufficiency but, although careful ophthalmoscopic examinations were made of its lenses, no change from the normal was detected. This would indicate that the lenticular degeneration described in this paper is due to parathyroid insufficiency and is not related to any thyroid insufficiency that might have been caused by the total thyroidectomy.

Goldmann (*loc. cit.*), in his study of experimental-tetany cataract in two dogs, has given us a most excellent account of the lenticular changes that occur after parathyroid removal. The following description of the lenses of the author's dog, P.T.N.S. No. 2, resembles quite closely the description that Goldmann made for his dog "Fifi." Because the writer was unable to make slitlamp examinations, the description given here will of course be less complete than that of Goldmann.

Examinations made during the first three to four weeks after parathyroidectomy failed to reveal any lenticular changes detectable by the methods used. At about the fourth week, when particular care was taken with a magnifying glass and transmitted light, it was noted that in the region of the anterior suture the individual lens fibers (or at least groups of a few fibers) could be made out. As the fibers approached the periphery, they became indistinct in outline, and in some cases could only be seen at points very close to the anterior suture lines. If the focal point rested on the posterior suture, the same phenomenon was presented. The sutures themselves were more or less indefinite. In about a week, it was estimated that the lens fibers themselves had become considerably more swollen and could be traced now to the pe-

riphery. The first detectable opaque points ("Punktrübungen") now appeared in small numbers and size in the subcapsular region, at the sutures, both anterior and posterior. It was not specifically noted that any "vacuoles" were present between the swollen lens fibers.

For purposes of comparison the description by Goldmann of the lenticular degeneration in his parathyroidectomized dog "Fifi" is given:

He states that the whole layer of "Punktrübungen" was "asbestglänzend" and was best examined with the magnifying glass and in transmitted light. When examinations were made in this manner, Goldmann found that in a careful examination he could make out the fiber diagram (which was not distinguishable in focalized light), which consisted of pure swollen fibers, whose course sprang from the anterior suture and continued to the posterior suture. These fibers lay in great tubes and only in the thin zone of vacuoles. The width of a single fiber was $1/24$ mm. by direct measurement (normal width about 0.01 mm.) If more attacks of tetany occurred, the vacuole opacities grew in size. Four months later, the layer of the older opacities had changed, the vacuole strands becoming finer, but now the fibers in focal light were fine-grained, opaque lines. This opaque layer was separated by a clear, subcapsular interval, giving a typical picture of zonular cataract. In the region of the opaque zone, was situated the suture which formed a Y-shaped slit. In transmitted light the fibers shone; the fundus reflex was now of the lower grade.

So far as the writer can determine, the lenticular changes as noted by him are almost identical with those described by Goldmann. There is perhaps only one particular difference; that is, in the eighth and ninth weeks after the parathyroidectomy was performed in the dogs in the present series, it was specifically noted that there was a marked increase in opaque points at both anterior and posterior sutures. The only change now and later on was that the lens fibers became more opaque and there was a decided increase, week by week, in the number and size of the opaque points at the sutures. Figure 1 illustrates the appearance of the right lens of this dog, 150 days after the operation, as seen with a magnifying glass and transmitted light. It may be compared with the illustration given by Goldmann for his dog "Fifi," one year

later than the description above, when Goldmann's animal showed the picture of zonular cataracts (Abb. 37, page 183).

As in Goldmann's case, it is easily seen that the lens fibers spring from the suture lines which form a "Y" at the anterior surface. In the case described by the writer and pictured in figure 1, the number and density of opaque points at the anterior suture were more pronounced, so that there a region of almost total opacity existed. For those unfamiliar with the lens-fiber diagram, a schematic drawing is shown in figure 2 illustrating the formation of the Y sutures at the anterior and posterior poles, with the direction of the lens fibers indicated. From this schematic drawing it may easily be seen wherein lies the source of the opaque lines representing the opaque lens fibers shown in figure 1.

In the one adult dog which lived long



Fig. 1 (Evans). Appearance of lens of right eye of young, parathyroidectomized dog (P.T.N.S. No. 2), 150 days after operation, as seen with a magnifying glass and transmitted light.

enough for lenticular changes to be evident, the pathology of the lens was not so spectacular. In this dog, a female, about 4 or 5 years old, one attack of tetany was observed, when the animal went into oestrus. The only change noted was the appearance of the subcapsular vacuoles, spherical in outline,

over the whole surface of the lens. This was true of both the anterior and posterior surfaces. These vacuole opacities appeared at about the third week after parathyroidectomy. The lenticular changes presented resemble somewhat those illustrated by Goldmann for his dog "Bobby," except that the vacu-

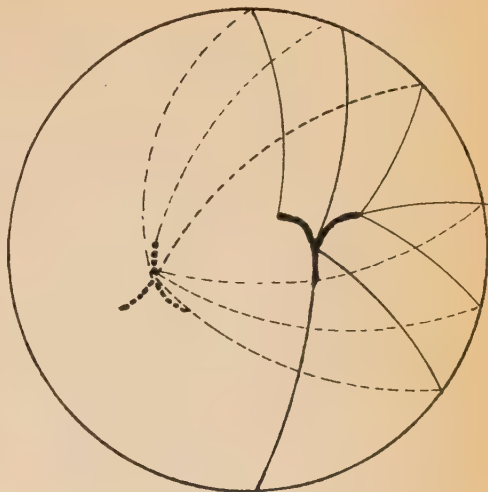


Fig. 2 (Evans). Schema illustrating the course of lens fibers from the anterior and posterior sutures (after Duke-Elder).

oles did not appear to be "comma shaped" but were more spherical in outline. It was never possible to make out the individual or grouped lens-fiber outlines in this animal. Up to the time of its death (57 days after the operation), the only detectable change in the lenses from the normal was the appearance of these opaque vacuoles, which tended to become more opaque in the seventh and eighth weeks after the operation. In figure 3 is illustrated the picture presented by this dog.

Discussion

At the outset of his work, Goldmann asked himself this question: How does cataract begin? What is its course of development, after parathyroid extirpation, from its beginning to its later form? and what is the relation of its development to the simple manifestations of tetany? It seems to the writer that there is given by Goldmann's and the author's researches at least a par-

tial answer to this question. It appears from both outlines of work that in the dog there is a distinct picture of the development and course of lenticular degeneration. The first manifestation seems to be a swelling of the lens fibers, especially in the young dog. Furthermore, the site of the first opaque points is in the region of the sutures. Before the writer had carefully read Goldmann's work, he was impressed with the fact that in the region of the sutures was to be found the greatest number of

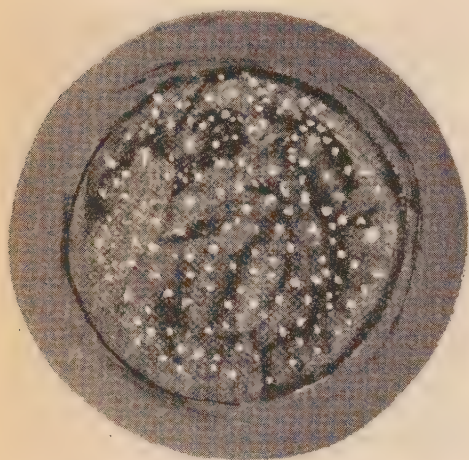


Fig. 3 (Evans). Appearance of right lens of adult dog (see text) as seen with the magnifying glass and transmitted light.

opaque points or, as Goldmann has expressed it, "The sutures are the site of predilection for opacities."

Just why the sutures should be the "site of predilection" for the appearance of the greatest number of opaque points is obscure. It seems probable, however, that they may be the regions of poorest nutrition, or they may be the points attacked by any hypothecated toxins. At least it is certain that in all of the five young parathyroidectomized dogs discussed in this paper, at the *suture* was to be found the greatest opacity.

Whether the development of "parathyroid cataract" in the human subject follows these general lines (swelling of individual lens fibers, opaque points first at the suture, etc.) seems not to have been determined (see O'Brien 1931). Goldmann made observations in

one case and found that the process in the dog was not like that in human cataract. It is necessary that closer observation be made of the general course of the development of postoperative parathyroid cataract in the human subject, especially soon after the initial signs of parathyroid deficiency are noted.

The relation of tetany to cataract

It would seem that there is offered in this study evidence that tetany, *per se*, plays no role in the development of parathyroid cataract. The older writers on the cause of cataract were impressed with the tetanic convulsions accompanying the lenticular changes and offered various explanations for the role of tetany in these changes. The principal theory was that the opacities were due to the ciliary-muscle spasms during the general tetanic seizures. Later workers saw the problem in a more reasonable light and believed that the cause of tetany and of the opacity was the same. They could not see how ciliary-muscle spasm alone could cause the opacity.

So far as the author can determine, these experiments offer conclusive evidence that the tetany which usually occurs during a state of parathyroid deficiency plays no role in the development of the cataract. These young dogs of the writer suffered the customary parathyroid deficiency as measured by biochemical changes in the blood, never experienced an attack of tetany, yet showed the characteristic lenticular degeneration. It is therefore evident that parathyroid cataract is due to other causes, some unknown, obscure change (toxemia) that takes place during the state of clinical parathyroid deficiency.

There is one important point in which the experiments of the writer are not in agreement with those made by Goldmann. The latter found that when tetany was not allowed to occur in dog "Bobby" (tetany was controlled by Luckhardt's method, 1.5 gms. calcium lactate per kilo each day), no further changes in the lens took place. In another, in which an attack of tetany was allowed later, new opaque points ap-

peared. The results led Goldmann to conclude that a state of acute parathyroid deficiency and tetany must intervene if lens changes are to occur. In the writer's experiments no calcium lactate was ever given, but tetany was controlled by the use of a proper milk diet. Nevertheless, though no tetany ever occurred (so far as the writer is aware), a similar lenticular change was noted in these dogs. At this time, the writer is unable to explain the discrepancy between Goldmann's and his experiments; i.e., cataract with and without tetany. The state of parathyroid deficiency in these animals and the efficacy of the sour-milk-bread diet used in these experiments has been discussed elsewhere and need not be repeated here.

Considerable attention has been paid in recent years to the possible role of the altered blood calcium in the development of parathyroid and naphthalene cataracts. Although it has been shown that the senile and experimentally produced parathyroid cataract both contain large amounts of calcium, it has by no means been proved that the increase in calcium content is the cause of the cataract. From the work of Salit (1933), it appears that large amounts of calcium are deposited in the cataractous lens only in the later stages and that in the "incipient stage" the calcium content of the lens is scarcely higher than in the normal lens. This suggests to the writer that calcification of the cataractous lens is the result of degenerative processes and is not the *cause* of cataract.

The toxin theory of parathyroid deficiency presented earlier by Luckhardt and by Dragstedt is not now in popular vogue because of the seemingly overwhelming evidence in favor of the calcium theory. Nevertheless, the writer believes that in our search for the cause of parathyroid and senile cataract, we should pay stricter attention to the possible role of toxins. It is difficult to understand how glutathione could have partially disappeared from these cataractous lenses unless toxins do play a role in parathyroid deficiency.

It would seem, too, that cataract developing in rats kept on low vitamin

G ("B₂") rations (Day and his coworkers, 1931) might well be explained by the "toxin theory." Although, in later years, this theory has had scant support, Chick (1933) has called attention to the possibility that pellagra may be caused by a toxic substance derived from a maize diet. Chick admits that the suggestion that pellagra may be caused by toxin derived from the diet (which can be corrected in the presence of certain foodstuffs, such as meat, milk, eggs, green vegetables, if given in sufficient quantities) is, at present, a speculation. It might nevertheless serve as a useful working hypothesis to those studying the etiology of pellagra, whether by the experimental or clinical method.

In a similar manner parathyroid cataract may well be caused by toxic substance brought about by faulty metabolism following removal of the parathyroids. Our findings on the partial disappearance of glutathione in parathyroid cataract would support this idea. It is an interesting fact recently expressed by Brand and Harris (1933) that the only three amino acids which seem to be available for purposes of detoxification in the human (glycine, glutamic acid, and cysteine) are the same as those which form glutathione. It is not surprising, then, that, if the toxin theory is correct, we should find this partial disappearance of glutathione from the crystalline lens in cataract. Perhaps in this case, too, glutathione is being removed in the process of detoxifying some now obscure substance, which is formed after the parathyroids are removed. This toxin theory of parathyroid deficiency in the etiology of parathyroid cataract is put forward by the writer in the hope that by its use researches in the etiology of cataract may be further stimulated. It may result in further progress toward the solution of the "mystery which at present shrouds the cause of the disease." Further, the general-toxin theory of parathyroid deficiency should be thoroughly tested (a thorough test has certainly never been made) before it is abandoned.

In acknowledgment, the writer wishes to express his thanks to Dr.

Peter Kronfeld for aid in the preparation of this manuscript, and to Dr. Arno B. Luckhardt for his unfailing assistance and encouragement in the carrying out of these researches.

Summary

Observations on the development of lenticular degeneration were made on five young and one adult parathyroidectomized dogs. These observations enabled the writer to conclude: (1) there is a characteristic change of the lens after parathyroidectomy in the young dog. The changes noted by the

writer were strikingly similar to those described by Goldmann. (2) As Goldmann has expressed it, in the parathyroidectomized young dog "the sutures are the site of predilection for opacities." (3) These opacities may occur in parathyroidectomized dogs in which the tetany is controlled by a sour milk-bread-liver-diet. Additional support is added to the view that tetany, *per se*, is not the cause of the cataract. (4) It is suggested that stricter attention be devoted to the possible role of "toxins" in parathyroid cataract.

University of Chicago

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7239 C

Studies on the Crystalline Lens. Fate of Glutathione in Parathyroid
Cataract.

EVERETTE I. EVANS. (Introduced by A. B. Luckhardt.)

From the Department of Physiology, University of Chicago.

It is well established that in senile cataract two of the principal biochemical changes are (1) a great increase of calcium and (2) an apparent total loss of glutathione.¹ Evans and Kern² demonstrated that in experimentally produced parathyroid cataract (by extirpation of the parathyroid glands), the calcium content of these cataractous lenses increased to approximately 25 times the amount present in normal dog lens.

It is probable that glutathione plays a definite rôle in the internal oxidation-reduction and enzymic reactions of the lens (initially suggested by Goldschmidt³). We were therefore naturally interested to learn the fate of the glutathione of the crystalline lens after removal of the parathyroids. Preliminary study indicated that the normal dog lens contains approximately 0.385% reduced glutathione, and that any figure considerably below this might be considered as indicating a loss of glutathione from the lens. The opportunity to study the fate of glutathione in parathyroid cataract was afforded by the early development of bilateral lenticular opacities in 5 young dogs (2 mos. old) that had been thyroparathyroidectomized in connection with another study. A complete description of these lenticular changes has been made elsewhere.⁴

At the time the dogs were sacrificed because of severe distemper,

¹ See discussion in Duke-Elder's Textbook of Ophthalmology, London, Henry Kimpton, 1932, p. 481.

² Evans, E. I., and Kern, R., *Am. J. Ophth.*, 1931, **14**, 1029.

³ Goldschmidt, M., *Arch. f. Ophth.*, 1924, **113**, 160.

⁴ Evans, E. I., *Am. J. Ophth.*, in press.

they had been without parathyroids for 57-152 days. The earliest signs of lenticular degeneration were evident at about the 25th day. The dogs were kept on a sour milk-bread-liver diet; tetany was not observed.⁵ The dogs were sacrificed by the intravenous injection of small amounts of sodium cyanide. Immediately after death the eyes were excised, the crystalline lenses removed and weighed on a watch glass.

Glutathione determination: Reduced glutathione was determined by the Okuda Method as modified by Hess⁶ for blood. The 2 lenses were ground with 10 cc. 10% sulphosalicylic acid and filtered by suction through hard paper. The protein residue was reground twice with 5 cc. portions of the same acid and the cake washed with two 2 cc. portions of the acid. The combined filtrates were made up to 30 cc. and an aliquot taken for immediate titration with M/1200 KIO₃ under conditions as specified by Okuda.⁷ For determination of oxidized glutathione, an aliquot was reduced with zinc and HCl. In 2 cases an aliquot was hydrolyzed with 20% HCl for 6 hours and the liberated cystine determined by the Sullivan Method.⁸ Also, in 3 cases, a Sullivan test for free cystine or cysteine was made directly on an aliquot of the sulphosalicylic acid extract. These data and other unpublished data (in collaboration with Drs. Sullivan and Hess) on glutathione in the crystalline lens indicate that the Okuda Method, when used on the sulphosalicylic acid extracts of the crystalline lens, titrates only glutathione of the reducing substances present.

TABLE I.
Glutathione in the cataractous lenses.

Dog No.	Days alive after operation	Free cysteine or cystine	Reduced Glutathione %	Oxidized Glutathione %	Total Glutathione %
1	57	-----	0.115	-----	0.115
2	82	-----	0.132	0.021	0.153
3	142	None	0.110	0.009	0.119
4	138	"	0.184	0.014	0.198
5	152	"	0.121	0.015	0.137
6-15*	-----	"	0.385	0.007	0.392
16-23†	-----	"	0.391	0.002	0.393

* 10 normal adult dogs.

† 8 normal young dogs.

Summary. The data shown in Table I indicate that before any appreciable calcium deposition takes place in the developing "para-

⁵ Evans, E. I., *Am. J. Physiol.*, 1933, **105**, 32.

⁶ Hess, W. C., *J. Wash. Acad. Sci.*, 1929, **19**, 419.

⁷ Okuda, Y., *J. Dept. Agri., Kyushu Imp. Univ.*, 1929, **2**, 133.

⁸ Sullivan, M. X., and Hess, W. C., *U. S. P. H. S., Public Health Reports*, Suppl. No. 86, 1930.

thyroid cataract" there is a serious loss of glutathione from the lens, amounting in the cases described here, from 53 to 72% of the amount present normally. Also, the negative Sullivan reaction for the lens filtrates indicates that free cystine is not the end-product of the destroyed glutathione.

If glutathione is as necessary for certain enzymic reactions and internal autoxidations as it is held by many to be, it is very probable that its removal or destruction must seriously impair the metabolism of the lens. Just how glutathione is destroyed after removal of the parathyroids is not clear but it may not be impertinent to suggest that it might be removed with glutathione from the other tissues, to detoxify certain injurious metabolic products that may arise after parathyroidectomy. Each of the 3 amino acids, glycine, glutamic acid, and cysteine are important detoxifying agents.⁹

These data on glutathione in parathyroid cataract suggest further the possible rôle of parathyroid deficiency (even in the absence of any tetany) in the pathogenesis of senile cataract.

⁹ Brand, E., and Harris, M. M., *Science*, 1933, **77**, 589.

